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In the footsteps of Darwin: dwarf male attachment sites in scalpellid barnacles (Crustacea: Cirripedia: Thoracica) – implications for phylogeny and the evolution of sexual systems

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Since Darwin, the presence of dwarf males in cirripedes has received much attention when trying to understand the evolution of reproductive strategies. The Scalpellidae is a large and monophyletic family of pedunculated barnacles, where the males always attach in confined areas (receptacles) on the rim of the mantle cavity. We used a broad range of microscopic techniques to describe the morphology of the dwarf male receptacle area in 11 species of scalpellids. We correlated this with their diverse habitats and reproductive biology. The purpose was to search for preformations in the receptacle that could assist male settlement and provide the female/hermaphrodite with control over the number eventually to be carried. The species fall into three groups based on their species-specific receptacle morphology and level of preformation. We concluded that small-sized, short-lived scalpellids occupying ephemeral substrata tend to invest more resources in caring for very few males. In contrast, large and long-lived species continuously acquire new males throughout their lifetime without investing any resources in their care. Thus, ecologically different habitats have directed the evolution of different mating systems. We discuss our findings in the light of a recent phylogenetical framework and the evolution of sexual systems.

ADDITIONAL KEYWORDS: cirripede – Darwin – dwarf males – ontogeny – reproduction – receptacle – Scalpellidae – sexual evolution.

INTRODUCTION

Barnacles (Cirripedia) display an extraordinary array of ecological and morphological diversity, which first prompted Darwin (1851, 1854) to use the group as ‘model organisms’ for his theories on natural selection. Ever since, cirripedes have attracted special attention as promising model species from both theoretical and experimental approaches, which, in particular,

have addressed questions on the evolution of reproductive systems (e.g. Charnov, 1982, 1987; Høeg, 1995; Yamaguchi *et al.*, 2007, 2012, 2014; Kelly & Sandford, 2010; Hoch & Levington, 2012; Yusa *et al.*, 2012; Høeg, Yusa & Dreyer, 2016).

The reproductive systems of cirripedes are interesting because they comprise pure hermaphroditism, androdioecy (coexistence of hermaphrodites and males) and dioecy (separate sexes). Further, cirripede males are always minute (dwarf males) and permanently attached to their much larger female or

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hermaphrodite partner (Klepal, 1987; Lin *et al.*, 2015; Dreyer *et al.*, 2018). Historically, Darwin (1851, 1854) was the first to elaborate on the structures on which the dwarf males attach to their partner. He noticed that cirripedes often had their males attached, usually several together, to the edges or base of the two scutal shell plates. Because the structures differed between species, and some species had this structure preformed as a depression in the shell plates prior to the arrival of dwarf males, he concluded with astonishing insight that the attachment site is purely an adaption to the reception of the dwarf male.

Recent studies have now demonstrated that the vast variations in the sexual biology of the Cirripedia can occur even at low taxonomic levels, such as in the pedunculated family Scalpellidae. This group comprises 250+ species and offers a particularly attractive theatre for studying the evolution of sexual systems for a variety of reasons. First, the group is monophyletic (Gale, 2016). Second, sporting all three reproductive systems mentioned above, its numerous species occur globally in highly diverse habitats, from shallow-water reefs to deep-sea ridges, in all cases being attached to a variety of both physical and biotic substrata (Buhl-Mortensen & Høeg, 2006, 2013; Yusa *et al.*, 2012; Lin *et al.*, 2015). Third, being nothing but a mere functional testis, scalpellid dwarf males have a highly specialized morphology, which bears little resemblance to their female or hermaphrodite partner, always lacking a vestigial canal and a mouth apparatus (Klepal, 1987; Dreyer *et al.*, 2017, 2018). They are, in the few investigated species, attached in morphologically confined areas, called receptacles, along the rim of the mantle cavity (Fig. 1; Darwin, 1851, 1854; Svane, 1986; Buhl-Mortensen & Høeg, 2006; Spremberg *et al.*, 2012; Gale, 2016; Dreyer *et al.*, 2018), from which mating and the subsequent fertilization of embryos is facilitated (Dreyer *et al.*, 2017). Most studies on scalpellid dwarf males have focused rather narrowly on the morphology of the dwarf males, which display various levels of reductions or specializations (Klepal, 1987). Unfortunately, the number of dwarf males, their precise location and the structure of their attachment sites have until now received very little attention in scalpellid barnacles (Darwin, 1851, 1854; Buhl-Mortensen & Høeg, 2006, 2013; Ozaki *et al.*, 2008; Spremberg *et al.*, 2012; Lin *et al.*, 2015; Gale, 2016). For a broader understanding of scalpellid sexual biology it would be important to also study the degree to which the female/hermaphrodite is specialized to receive the males. Such data could be particularly valuable in correlations with the general life-history patterns of the species. Recent molecularly and morphologically based phylogenies of the Scalpellidae now offer a robust framework on to which we can project both structural

and biological information from both extant and fossil species (Lin *et al.*, 2015; Gale, 2016).

Walking in the footsteps of Darwin, we here analyse the biology of the dwarf male settlement site in 11 species of scalpellid cirripedes differing in size, mating strategies and general ecology. We (1) study the morphological diversity of scalpellid receptacles based on a new, standardized terminology, using light microscopy (LM), scanning electron microscopy (SEM) and micro-CT-scanning (μ -CT). Based on this, we then classify the receptacles into morphological groups and analyse, by statistical methods, whether these groups are correlated with reproductive data and life-history traits of the species in terms of the size of the females and hermaphrodites, their microhabitat and the number and size of their associated dwarf males. From here, we (3) follow the ontogeny of receptacles in three selected species in order to decide if they are preformed in a way that benefits the dwarf males and enables the females or hermaphrodites to control their actual numbers. We project these results on recent phylogenetic work and discuss the evolutionary pathways that receptacle morphologies might have taken. In the light of this, we discuss how this can contribute to a general understanding of the evolution of reproductive strategies and sexual systems.

MATERIALS AND METHODS

MATERIALS

We studied 11 species of scalpellid barnacles from several samples of various museums and field collections. These are listed in Table 1. The species were carefully selected to represent the morphological diversity of the family. Naming of species is in accord with Gale (2016).

MICROSCOPY AND EXAMINATION OF SPECIMENS

Light microscopy (LM)

Specimens were photographed in ethanol with a Leica MZ dissection microscope using either an Evolution or macro optics of an Olympus C5050 camera.

Scanning electron microscopy (SEM)

The receptacle area (the scutum and the accompanying tissue, see Terminology-section) was dissected free from the chosen specimens and rinsed carefully with demineralized water. The shell plates used for digestion studies were then transferred to a Danish commercial 0.5% bleach solution for 1–4 h, and subsequently rinsed in demineralized water and air dried before mounting for SEM. Specimens used intact were from demineralized water dehydrated in

Table 1. List of species studied with details on museums, sample ID, collection depth, site and year, and number of species used in the study

Species	Museum	Sample	Collection details	Number of specimens examined
<i>Scalpellum stearnsii</i> (Pilsbry, 1980)	Muséum Nationale d'Histoire Naturelle (MNHN), Paris, France	Ci-1815	569–595 m, Phillippine Sea, Isla Han Samar, Philippines	103
<i>Regioscalpellum regium</i> <i>Trianguloscalpellum regium</i> (Wyville Thomson, 1873)	Muséum National D'Histoire Naturelle (MNHN), Paris, France	Ci-2266	2225–4844 m, Mid Atlantic Ridge, Fracture Zone of Kane	30
<i>Arcoscalpellum michelloti-anum</i> (Sequenza, 1876)	Muséum D'Histoire Naturelle (MNHN), Paris, France	Ci-2870, Ci- 2676 Ci-2075	3134 m, Atlantic Ocean, Gulf of Cadiz, Portugal, 1984	27
<i>Scalpellum scalpellum</i> (Linnaeus, 1767)	Det Naturhistoriska Riksmuseet, Stockholm, Sweden	NRM-890 NRM-980	30–60 m, around Vadeöerna, Sweden, 1887	327 + 8 for ontogeny
<i>Teloscalpellum antillarum</i> (Pilsbry, 1907)	Smithsonian Institute (SI), Washington, USA	UNSM-SI-1022335	2930–3030 m, North Atlantic Ocean, Gulf of Mexico	29
<i>Amigdoscalpellum rigidum</i> (Aurivillius, 1898)	Muséum D'Histoire Naturelle (MNHN), Paris, France	Ci 2592, Ci 2590, Ci 2625, Ci 2647	2183–2158 m, Atlantic Ocean, Acores, 1996	9
<i>Weltnerium nymphocola</i> (Hoek, 1883)	Museum für Naturkunde, Berlin, Germany	22251	588 m, North Atlantic Ocean, west coast of Bergen, Norway, 1922	8
<i>Weltnerium gibberum</i> <i>Ornatoscalpellum gibberum</i> (Aurivillius, 1898)	Smithsonian Institute (SI), Washington, United States of America	USNM-SI-125287	106–110 m, South Atlantic Ocean, Tierra del Fuego, Argentina, 1962	227 + 6 for ontogeny
<i>Weltnerium stroemii</i> <i>Ornatoscalpellum stroemii</i> (M. Sars, 1859)	Bergen Museum (BM), Zoological Collection, Norway	30538	350 m, North Atlantic Ocean, west coast of Bergen, Norway, 1902	18 + 6 for ontogeny
<i>Verum brachiumcancrici</i> (Weltner, 1922)	Museum für Naturkunde, Berlin, Germany	22257, 22258, 22261	500 m, south Atlantic Ocean, South Africa, 1922	6
<i>Anguloscalpellum angulare</i> (Nilsson-Cantell, 1930)	Smithsonian Institute (SI), Washington, USA	USNM-SI-125246	128–16 m, Antarctic Ocean in 1962	24

a graded alcohol series and critical point dried using liquid CO₂. All specimens were mounted with double adhesive tape, sputter coated with palladium in argon and studied in a JEOL-JSM-6335K scanning electron microscope. Photo plates were constructed using COREL DRAW.

Micro-CT-scanning (μ-CT)

Prior to each micro-CT-scanning, each specimen was stained overnight in Lugol's solution, then mounted on a holder made of tightly fitting plastic vials. A Phoenix Nanotom (GE Sensing & Inspection Technologies) cone beam CT-scanner located at the Bavarian State

Collection of Zoology, Munich, was operated at a voltage of either 140 kV and a current of 120 μ A or of 100 kV/70 μ A for 54 min each. A total of 1440 radiographs were registered in each scan and saved as TIFF stacks for processing in DRISHTI before being arranged and edited in COREL DRAW.

REPRODUCTIVE DATA AND STATISTICAL ANALYSES

We measured the size of the specimens as the length of the capitulum, since the peduncle can change in size and as fixation conditions vary between similar-sized individuals. The numbers of attached dwarf males were scored on both left and right sides of the scutal plates. Total male numbers include unmetamorphosed cyprids attached in the receptacle areas but not debris left from dead ones. We also measured, as accurately as possible, the maximum length of the adult dwarf males in all investigated species. To this we added existing reproductive data on some of the species investigated (Kaufmann, 1965; Buhl-Mortensen & Høeg, 2006, 2013; Ozaki *et al.*, 2008; Spremberg *et al.*, 2012).

We statistically analysed the relationships between receptacle morphology and traits of females/hermaphrodites and dwarf males among the 11 species using JMP v.11 (SAS Institute, Cary, North Carolina). Phylogenetic information was not incorporated as no phylogenetic tree containing all these species was available (see Lin *et al.*, 2015; Gale, 2016). The relationship between female/hermaphrodite size and receptacle types was investigated using a multinomial logistic regression. For the other analyses, generalized linear models (GLMs) with Gaussian errors and a unity link were used. Overdispersion was not detected in any test except for the relationship between receptacle type and number of males. In the latter case, the overdispersion parameter was taken into consideration as implemented by JMP.

TERMINOLOGY

We classified juveniles as all specimens of females/hermaphrodites smaller than the minimum size where males were found. This size differed between the species. For *S. scalpellum* and *W. stroemii*, we relied on the analysis based on large samples presented by Buhl-Mortensen & Høeg (2006). For *W. gibberum*, we used our collection of 200+ specimens. Adult females or hermaphrodites were considered 'virgin', when they carried embryos but no males and showed no signs in the tissue, such as degenerating males or scars, of having previously had any males. The top of the capitulum is considered 'apical' (or 'posterior') and the attachment site 'basal' (morphologically 'anterior'). The carinal edge of the capitulum is 'dorsal' and the mantle aperture 'ventral'.

RESULTS

Using *S. scalpellum* as an example, Figures 1 and 2 summarize the terminology we use and provide a morphological overview of the receptacle in scalpellid barnacles. They show how cypris larvae settle and metamorphose into dwarf males in relation to the receptacle morphology.

GENERAL STRUCTURE AND POSITION OF THE MALE SETTLEMENT AREA

Functionally, we define the receptacle as the area on the rim of the mantle cavity where dwarf males are found attached on the female or hermaphrodite partner (Figs 1, 2). In all but one species, we invariably found two such areas located symmetrically on either side of the on the scutal plates (Figs 1, 2B). The precise location, size and shape of the receptacle area varied with species. Only in very exceptional cases were settled cyprids or metamorphosed dwarf males found elsewhere along the mantle rim (Spremberg *et al.*, 2012; Høeg *et al.*, 2016). In specimens where no males have yet settled, there are no clear morphological characteristics in external morphology that identify the receptacle area. In laboratory trials where many cyprids can settle near simultaneously, attachment takes place in these two perfectly symmetrically placed areas (Spremberg *et al.*, 2012; Dreyer *et al.*, 2018; Figs 1, 2B).

When viewed face on, the mantle rim is the narrow, cuticle and seta-covered area between the mantle cavity proper and the inner edges of the opercular plates (the paired scuta and terga; Figs 1C, 2C, D). The transition between the mantle rim and the mantle cavity is marked by a thin, cuticular membrane, the ventral transparent lamellae (the vtl; Spremberg *et al.*, 2012; Figs 1B–E, 2B–D), which runs along the entire mantle aperture. Cyprids developing into dwarf males invariably settle on the cuticle covering the mantle rim and never on the shell plates themselves or in the mantle cavity proper, i.e. inside the ventral transparent lamellae (Figs 1, 2). The cuticle covering the mantle rim between the vtl and the inner edges of the opercular plates is not shed during the moulting process (Blomsterberg *et al.*, 2004). This entails that cyprids settling in this area can remain attached as dwarf males when the cuticle of the mantle cavity is shed, such as occurs in all cirripedes between successive broods (Anderson, 1994). Being located on the rim of the mantle outside of the vtl, the dwarf males are in principle exposed to the exterior when the mantle valves are closed. But in *W. stroemii*, *W. gibberum*, *W. nymphocola* and *V. brachiumcancrini*, the structural morphology of the mantle rim in the receptacle area means that the males are functionally on the inside of the mantle cavity when the

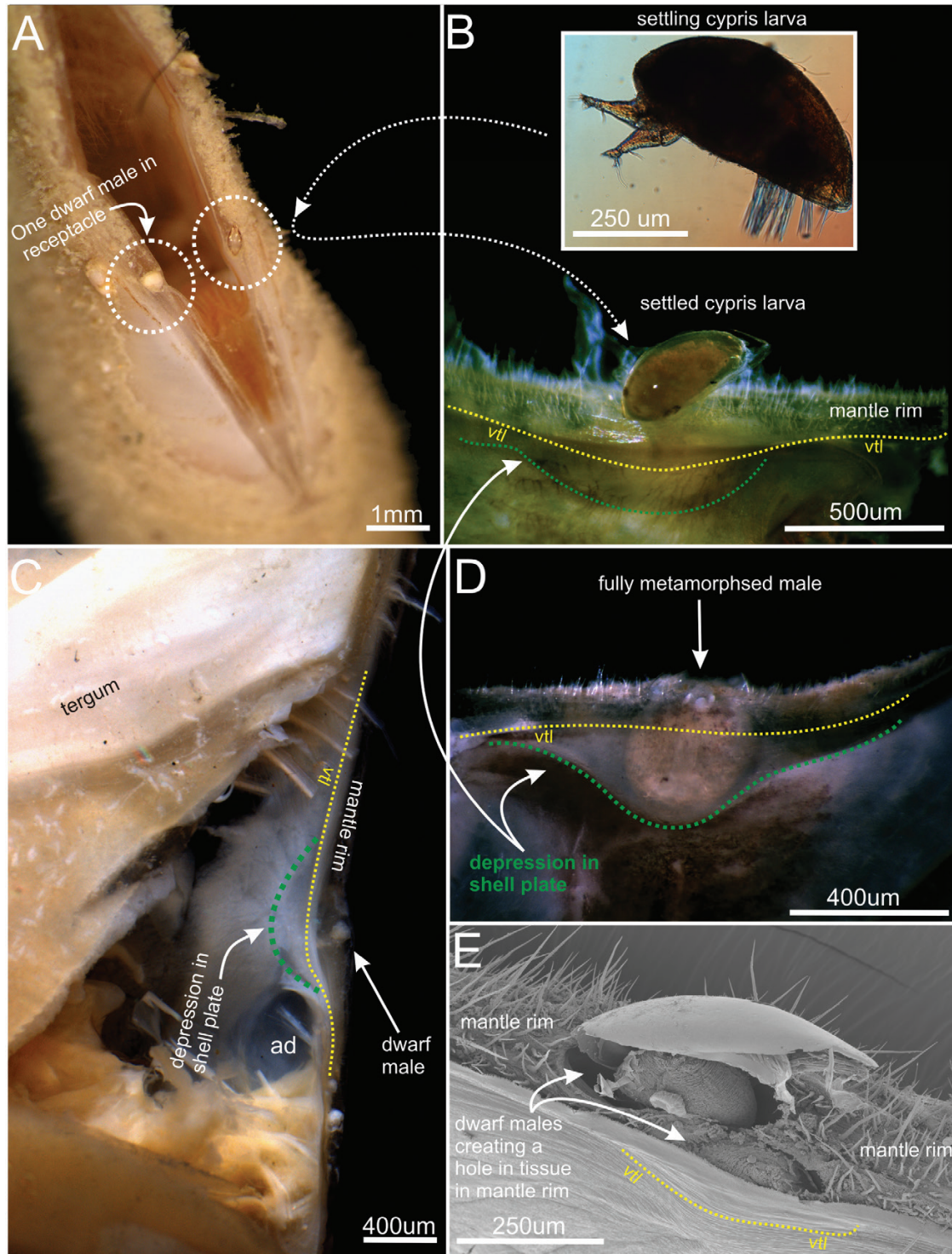


Figure 1. Settlement, development of larvae and generalized receptacle morphology in *Scalpellum scalpellum*. A, a hermaphrodite with a fully developed dwarf male (left receptacle) and a newly settled cypris larva. B, a free-swimming and settling cypris larva with its antennules extended anteriorly (insert) and a newly settled cypris larva in the receptacle area with underlying shell plate. C, medial view of the receptacle area in right receptacle side with one fully developed dwarf male on top of the depression in the shell plate. D, a fully developed male deeply buried in the receptacle. E, SEM of two dwarf males settled in the receptacle area. One (right most) is more developed and buried deeper. Abbreviations: ad, adductor muscle; vtl, ventral transparent lamellae.

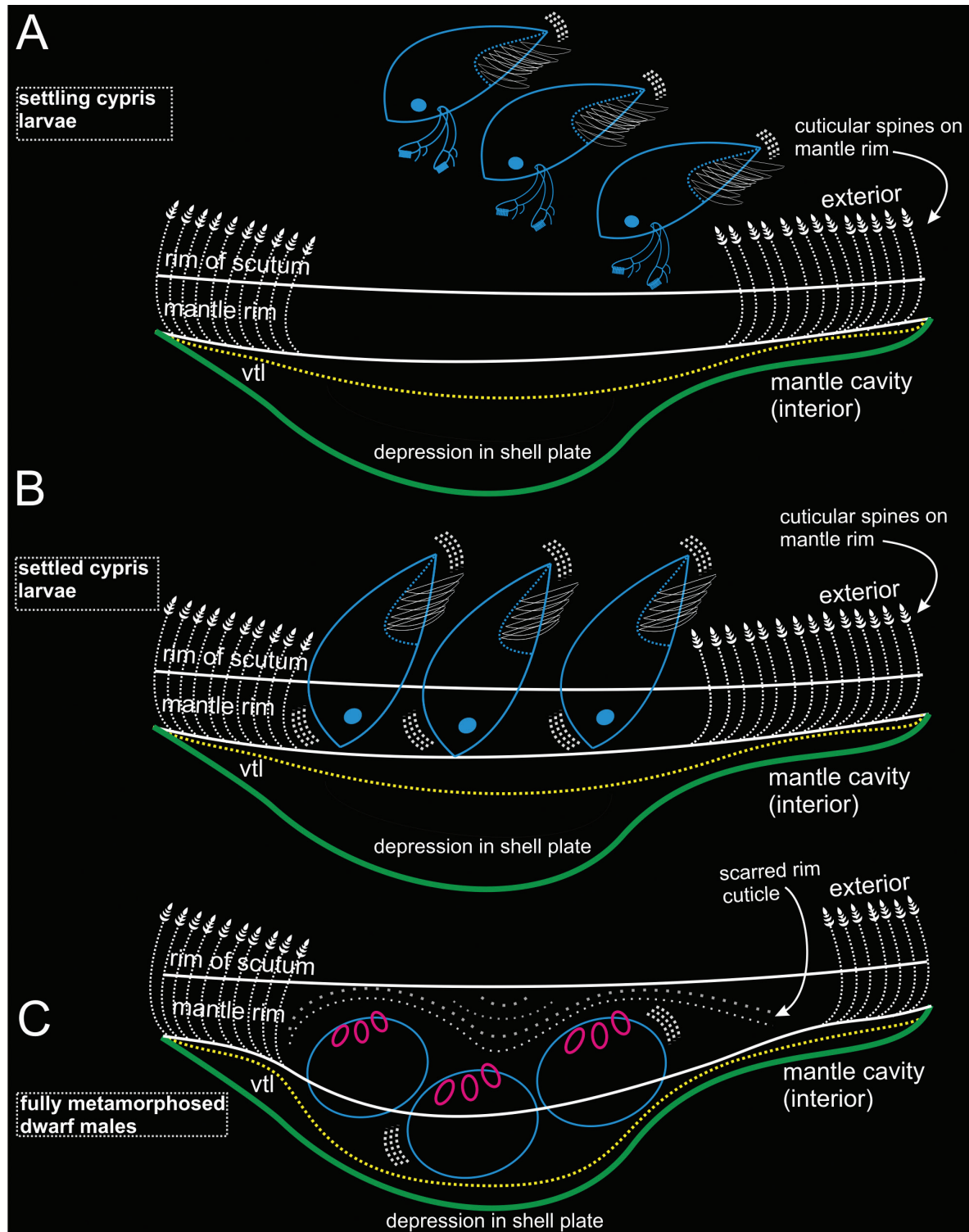


Figure 2. Schematic overview of settlement, development of larvae and generalized receptacle morphology in *Scalpellum scalpellum*. A, free-swimming cypris larvae with antennules extended settle in the receptacle. B, newly settled cyprids in the receptacle directly on the mantle rim cuticle. No change in the external morphology of receptacle. C, the settled cyprids have metamorphosed to smaller dwarf males. The mantle rim has been scarred in the cuticle from burrowing dwarf males. Note the medial curving of the ventral transparent lamellae after development of males. Abbreviation: vtl, ventral transparent lamellae.

valves are closed (Figs 6D, 7G, 8E, F). In these species, inspection for dwarf males, therefore, involves that the mantle valves are forced apart. The vtl always curve slightly inwards in the receptacle area (Fig. 1). This entails that the mantle rim here is somewhat broader, thus yielding more space for settlement. The settlement and development of dwarf males sometimes push the pliable lamellae even further inwards towards the mantle cavity, especially in the smaller sized species. The inward curvature of the vtl is less pronounced in juvenile specimens and in virgin adults without dwarf males. The subsequent development of dwarf males might also in some cases broaden the mantle rim area as seen in the larger species (Fig. 3G).

RECEPTACLE MORPHOLOGY IN SINGLE SPECIES

Based on the investigations below, we divide the scalpellid receptacles into three groups, which have been summarized in Table 2 (also see Discussion). In seven of the 11 investigated species, we studied both soft tissue features, including the numbers and position of males, and the morphology of the shell plates after tissue digestion. For three species, we also had sufficient numbers of small-sized specimens to establish the minimum adult size and to later on follow the ontogeny of the receptacle area in the juveniles. In two species, we had so few specimens, or they were in such a poor condition, that they were studied only as tissue-digested shell plates in SEM.

Table 2. Summary of sexual systems, receptacle groups and reproductive data in the ten examined species of Scalpellidae. Primarily originating from own samples but also from Buhl-Mortensen & Høeg (2006, 2013) and Spremberg *et al.* (2012). Abbreviations: D, dioecy; AD, androdioecy

Species	Sexual system	Group	Adult size (mm)	Male size (µm)	Depth range (m)	Substratum	# males per receptacle
<i>S. stearnsii</i>	D	1	29.0–61.5 (Avg., 38.3)	380–880 (Avg., 650)	380–595	Mollusc shells and debris	Many 0–100 (Avg., 27.5)
<i>R. regium</i>	D	1	15.0–35.0 (Avg., 25.0)	1000–1500 (Avg., 1300)	1507–6135	Mollusc shells, sea mounts	Many 0–30 (Avg., 27.0)
<i>A. michellotianum</i>	D	1	41.2–43.9 (Avg., 42.5)	1500–1600 (Avg., 1450)	64–5190	Gorgonians	Many 0–40 (Avg., 25)
<i>S. scalpellum</i>	AD	2	7.0–22.0 (Avg., 14.5)	300–550 (Avg., 440)	45–100	Mostly hydroids and polychaete tubes	Intermediate 0–13 (Avg., 2.5)
<i>T. antillarum</i>	D	2	12.0–14.7 (Avg., 12.5)	850–900 (Avg., 850)	351–3850	Unidentified spines	Intermediate 0–8 (Avg., 6.4)
<i>A. rigidum</i>	D	2	20.0–22.0 (Avg., 21.5)	700–900 (Avg., 860)	2047–3590	Anthozoans	Intermediate 0–13 (Avg., 13)
<i>W. nymphocola</i>	D	3	8.0–9.0 (Avg., 8.5)	700–760 (Avg., 700)	28–1358	Pycnogonids	Very few 2 (Avg., 2)
<i>W. gibberum</i>	D	3	7.0–11.0 (Avg., 9.4)	600–850 (Avg., 750)	53–132	Gorgonians, hydroids	Very few 2 (Avg., 2)
<i>W. stroemii</i>	D	3	5.0–10.0 (Avg., 7.0)	750–900 (Avg., 800)	100	Gorgonians, hydroids	Very few 2 (Avg., 2)
<i>V. brachiumcancricri</i>	D	3	6.9–10.5 (Avg., 10.54)	700–770 (Avg., 750)	500–600	Decapods	Very Few 2 (Avg., 2)
<i>A. angulare</i>	D	3	1.8–3.9 (Avg., 2.89)	700–800 (Avg., 770)	100–200	Unidentified spines	Very few 2 (Avg., 2)

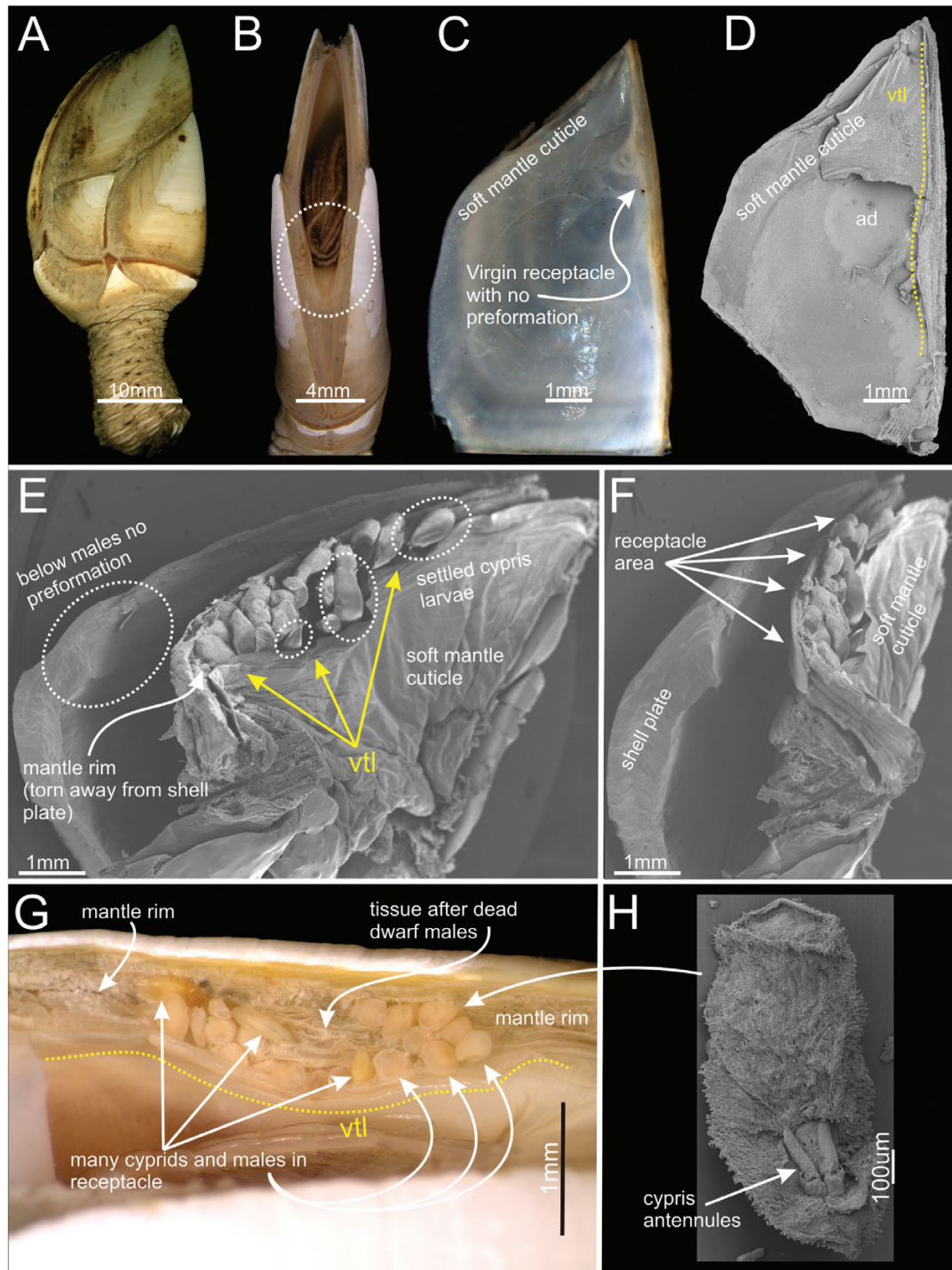


Figure 3. Receptacle morphology in *Regioscalpellum regium*. A, overview of adult specimen before dissection. B, ventral view of receptacle area with many males (circled). C, scutum of juvenile specimen with no external preformation for hosting later arriving dwarf males. D, SEM of (C) with the ventral transparent lamella curving below the adductor muscle scar. E, SEM of receptacle area on dissected scutum with many males and newly settled cyprids. Note no preformation underlying the torn-apart mantle rim. Note also how the ventral transparent lamella curves more in the area with crowded males and cyprids. F, SEM of receptacle area in (E) with no preformation in the shell plate. G, LM of (E), with a large crevice in the receptacle forced by burrowing dwarf males. H, SEM of dwarf male with cuticular spines along the sides of the body. Anteriorly (bottom) is the remnants of cypris larval antennules used for attachment. Abbreviations: ad, adductor muscle; vtl, ventral transparent lamella.

Regioscalpellum regium

In this species, the receptacles are located rather close to the scutal adductor muscle and each can simultaneously hold up to 30 dwarf males (Fig. 3A, B, E–G). The same receptacle can host dwarf males of variable ages comprising recently settled cyprids, mature males, some that seem to be dead and degenerating, with holes left behind from males that have completely disappeared (Fig. 3E, G). The receptacle is a single, rather narrow but elongated crevice (Fig. 3E, F, G), at the bottom of which the individual dwarf males and cyprids are tightly crowded with no intervening cuticle or tissue, as seen in some other species (Fig. 3F). Perhaps due to crowding in the receptacle crevice, the cyprids and dwarf males have no preferred orientation relative to the female partner (Fig. 3E, F, G). We found no crevice in juveniles or adult, virgin specimens (Fig. 3C, D). Since the latter can potentially carry males, the absence of a crevice shows that this feature is not structurally preformed prior to arrival of the first males (Fig. 3C, D). SEM of specimens with the mantle rim torn away from the scutal shell plates failed to show any preformation in the shell plate below the receptacle area (Fig. 3E, F). The dwarf male is much smaller and elongated than its partner and bears no large, calcified shell plates. It is further equipped with many small, spinous cuticular structures (Fig. 3H).

Arcoscalpellum michelottianum

In adults, the receptacle morphology resembles the one described for *R. regium* and our results confirm and extend those results previously given by Buhl-Mortensen & Høeg (2006). Irrespective of specimen size, the mantle rim is broad with a thick layer of tissue, but we had too few juveniles to establish a minimum size for adults. As in *R. regium*, there is no visible crevice in the mantle rim tissue in adult, virgin specimens prior to male settlement, nor any structural preformation in the underlying scutal shell plate (Fig. 4B, C–E). The receptacle area is located apically to the scutal adductor, but not close to the transition to the tergum (Fig. 4A, B, D–G). The receptacle holds up to 20 males, and when several are present, they are all located in one single, deep and elongated crevice (Fig. 4D–F). The canyon-like crevice seems to be formed by individual males, each forming their own hole in the tissue, as seen in *S. stearnsii* (see below), but such holes later coalesce to one long crevice as numerous males accumulate in the area (Fig. 3E). In agreement, some specimens with a single male had no elongated crevice but only a hole slightly larger than the male itself (Fig. 3F, G). Males settling close to the adductor muscle seem unable to dig in as deep as those located further from this structure (Fig. 4D, G).

Scalpellum scalpellum

This species was studied by LM, SEM (Fig. 1) and μ -CT (Fig. 5) using both juveniles and adult specimens, with and without males. The following description also relies on our previous results on this species based on direct observations of larval settlement and behaviour in the laboratory (Spremborg *et al.*, 2012; Høeg *et al.*, 2016; Dreyer *et al.*, 2017). In both juveniles and adults of *Scalpellum scalpellum*, the mantle rim is slightly broader in the receptacle area apical to the scutal adductor muscle, but otherwise there is no external identification of this area when males are not present, e.g. no clearly visible depressions into which the cypris larvae may settle (Figs 1A, C, E, 5K). But μ -CT and SEM clearly revealed the presence of an elongated, shallow preformation in the shell plates underlying the receptacle area, both in juvenile specimens and in adults, irrespective of male presence or absence (Fig. 5E–I). This depression is also clearly seen in specimens with digested shell plates (Fig. 5B, C). In both SEM and μ -CT, the adult specimens, whether with males or virgins, have a more developed, deep and oval depression (Fig. 5C, D). Male numbers are moderate in *S. scalpellum* (Figs 1, 5; Table 2), but when many males occupy a single receptacle, some may be located so peripherally relative to the shell plate depression that they have been unable to dig in deep (see Spremborg *et al.*, 2012). Thus, while some males can be almost completely buried (Figs 1C, D, 5G, K, J), other males can occasionally be almost fully exposed.

Weltnerium nymphocola

Buhl-Mortensen & Høeg (2013) noted this species to be very similar to *V. brachiumcancri*, but we found considerable differences between the two species. The mantle rim is rather narrow in adult specimens and the scutal plates seem comparably thinner and narrower than in *V. brachiumcancri* (Fig. 6A, B). We had no access to juvenile specimens, but the receptacle area lies almost at the transition to the tergum (Fig. 6C, E). Below, there is a preformed depression in the scutum, which has a very distinct 'banana'-shape and a full length in excess of 500 μ m (Fig. 6C). The depression extends ventrally from the mantle rim almost to the dorsal end of the scutum (Fig. 6C, D), and it fits with the morphology, size and shape of the dwarf male, entailing that only a single male can fit into the receptacle (Fig. 6D, E). Thereby, the shell plate depression allows the elongated, sac-shaped male (Fig. 6E) to burrow itself very deep into the overlying tissue, whence only its rounded apical end is visible in external view (Fig. 6D). Although smaller, the dwarf male resembles the male of *R. regium* in external morphology, carrying no calcified shell plates and having cuticular structures projecting laterally from its body (Fig. 6E).

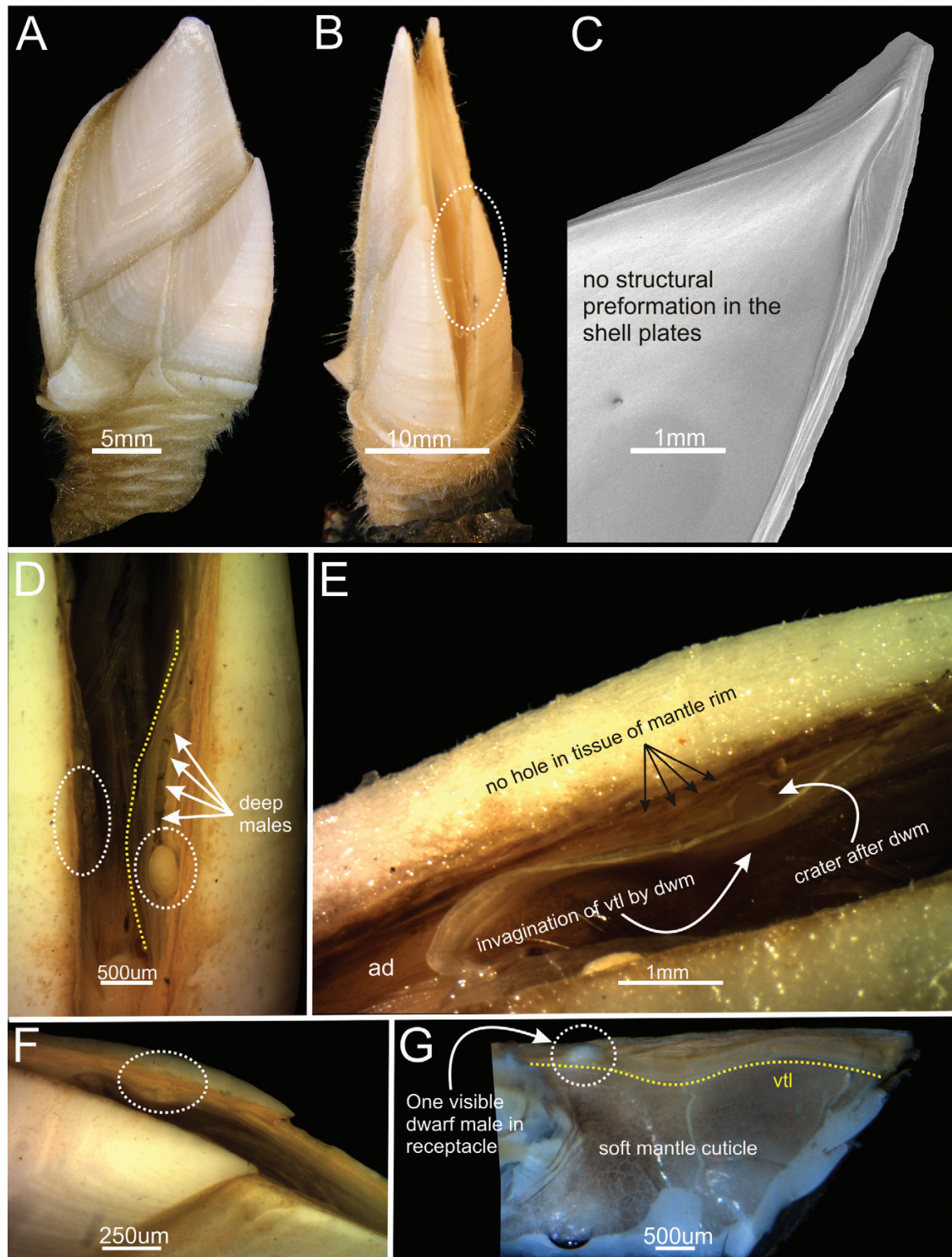


Figure 4. Receptacle morphology in *Arcoscalpellum michellotianum*. A, overview of adult specimen before dissection. B, ventral view on receptacle area with no males (circled). C, SEM of a digested scutal shell plate without preformation in receptacle area. D, ventral view of left and right receptacles with no males in left and a few in right. The male closest to the adductor muscle is more exposed due to spatial constraints in the tissue. E, close-up of (D), with crater after dwarf male leaving the ventral transparent lamella curved inwards. F, an adult specimen with a few males in the receptacle. G, medial view of adult with the receptacle inhabited by a single male. Abbreviations: ad, adductor muscle; vtl, ventral transparent lamella.

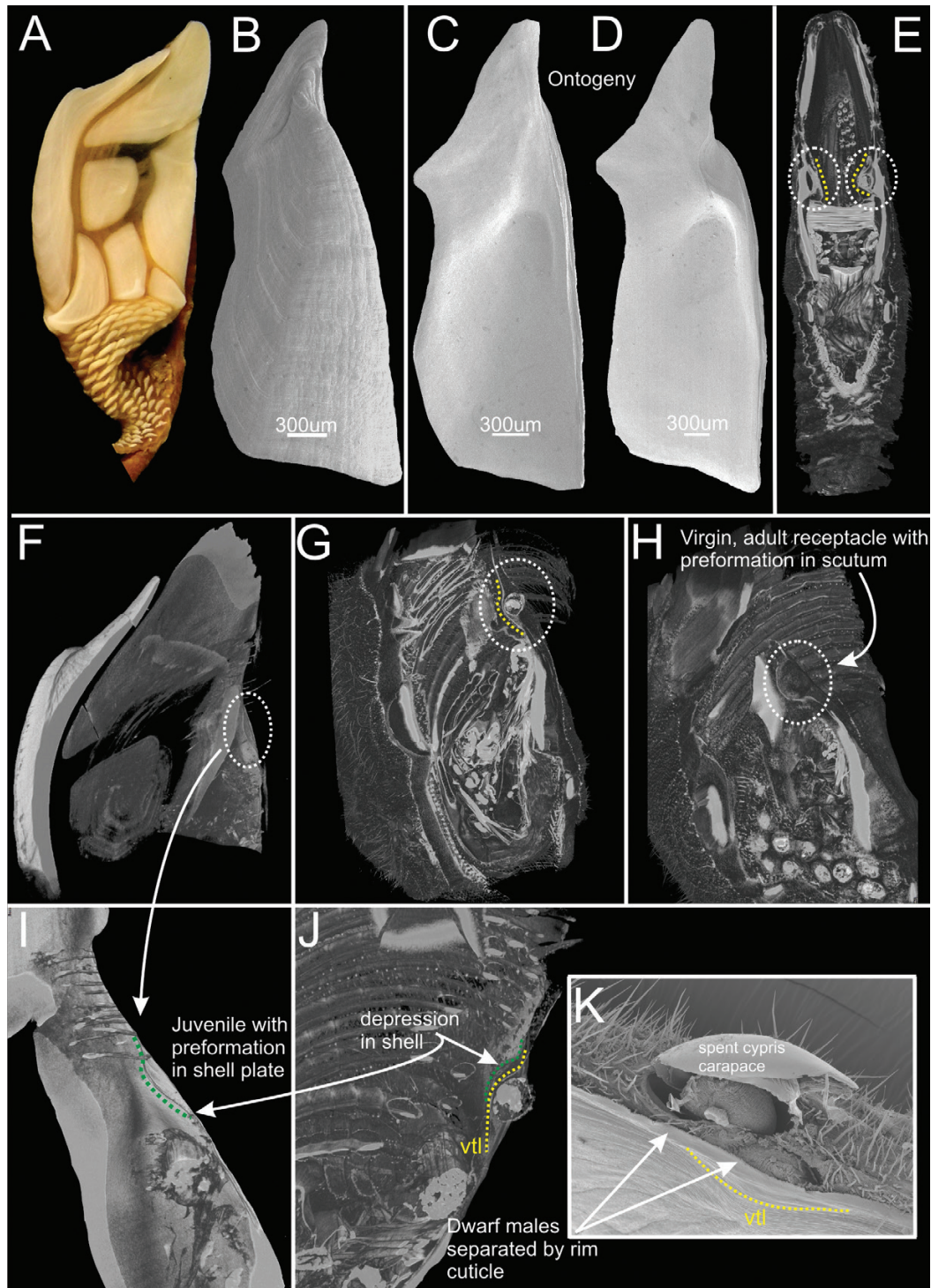


Figure 5. Receptacle morphology in *Scalpellum scalpellum*. A, B, overview of adult specimen before dissection and outer scutal shell plate morphology. C, D, SEM of ontogeny in juvenile (C: <7 mm) and adult (D: >7 mm) specimens. Note the change from a shallow preformation to a deeper, oval preformation. E, μ -CT of specimen in (A), with left receptacle without dwarf males and right with one deeply buried one. F, I, μ -CT of specimen in (C), before digestion of scutal shell plates. Note the same shallow preformation. G, J, μ -CT of right receptacle in (E), with a deeply buried male. H, μ -CT of a virgin, adult receptacle with the preformation underlying the mantle rim. K, SEM of settled dwarf males and the mantle rim cuticle intact elsewhere. Note that the carapace belongs to the former cypris larva. vtl, ventral transparent lamella.

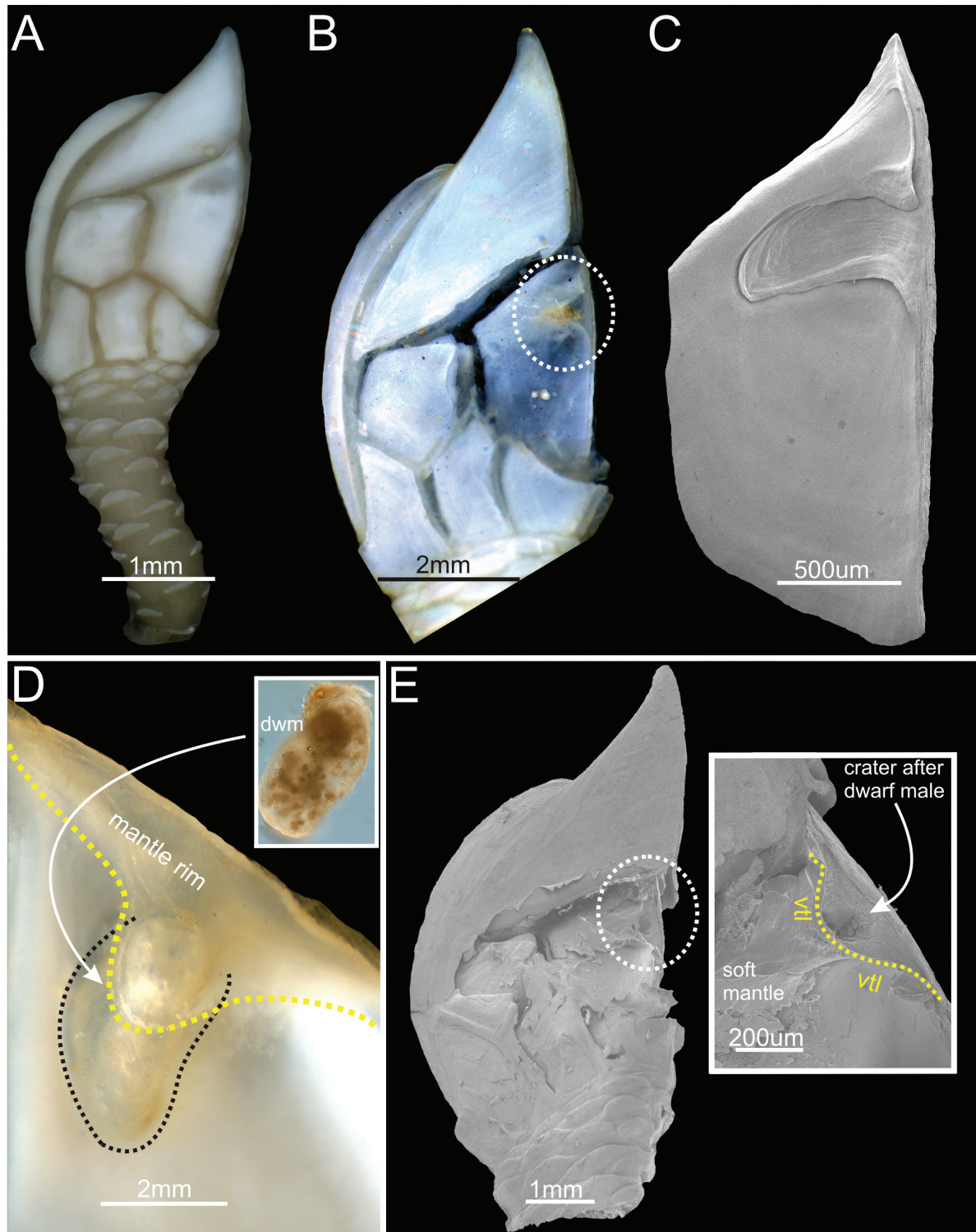


Figure 6. Receptacle morphology in *Weltnerium nymphocola*. A, overview of adult specimen before dissection and outer scutal shell plate morphology, with the depression in the scutal shell plate visible close to the transition to the tergum. B, close-up after dissection of left-side receptacle exposing the dwarf male in the right-side receptacle. C, SEM of digested scutal shell plate with a clear, distinct preformation close to the transition to the tergum. D, close-up of (B), with the dwarf male (insert) body fitting perfectly in the receptacle pocket. E, SEM of (B), with dwarf male dissected out exposing the crevice in the tissue it created while burrowing into the receptacle pocket. Abbreviations: dwm, dwarf male; vtl, ventral transparent lamella.

Verum brachiumcancreri

The receptacle area lies close to the transition to the tergum (Fig. 7A–D), where the mantle rim locally broadens even more than in *W. nymphocola*. In external view, the area looks similar before and after arrival of males (Fig. 7F, G). After settlement, the males bury themselves deep into the tissue and, as in *W. nymphocola*, they end up lying functionally inside the mantle cavity when the mantle valves are closed (Fig. 7F, G). Tissue-digested scutal plates showed the presence of a very specialized and preformed depression that fits only the size of a single male (Fig. 7D). The structural depression extends from the mantle rim towards the dorsal end of the scutum and has a complex shape, consisting of an outer, broader part and an inner, narrower part (Fig. 7D).

Weltnerium gibberum

Our material comprises more than 200 specimens, including both juveniles and adults. The receptacle area is situated almost at the transition to the tergum, where the mantle rim is slightly broader than elsewhere (Fig. 8A–E). Otherwise, there is no external identification of this area, both in juvenile and adult individuals, where dwarf males are not present (Fig. 8D). In contrast, SEM of digested scuta revealed the presence of a preformed depression, which is not in direct contact with the mantle rim (Fig. 8C). The depression has a sleeping bag-like shape and extends almost to the dorsal rim of the scutal plate (Fig. 8C). In all examined specimens, the pocket holds an apically located and minute hole-like structure of unknown origin and function. Due to the shape of the depression, only a single male can bury deep into the tissue and thus gain protection. In one receptacle area (Fig. 8B, E, F), we found that one cypris had settled next to an already settled dwarf male. This cypris was much more exposed than the adjacent male, illustrating that only the one that manages to settle first and directly over the shell plate depression will be able to bury itself deeper down in the receptacle pocket. Even deeply buried dwarf males have their roundish apex exposed on the mantle rim (Fig. 8F). We emphasize that such exposed males had a perfectly normal morphology and seemed to be fully functional.

Scalpellum stearnsii is exceptional because the dwarf males are not located in a confined area. Instead, the numerous males are sited in a row in the mantle rim tissue all along the edge of the scutal plate (Fig. 9A, C). The dwarf males are situated in individual pits in the tissue that seem never to fuse into larger cavities (Fig. 9C). There are many males (up to 100; see Ozaki *et al.*, 2008) and there is no structural preformation in the shell plates, as seen in the other large females of *R. regium* and *A. michellotianum* (Fig. 9B). In *Amigdoscalpellum rigidum* we found a shallow-sized depression in the shell plate under the receptacle area housing up to ten males at a time (Fig. 9E, F). This is located at the very edge of the transition to the tergum (Fig. 9D,

E). The area of the receptacle is approximately half the size of the settlement area of *R. regium* and *A. michellotianum* (Fig. 9E, F). In *Teloscalpellum antillarum* we also found a depression in the shell plate under the receptacle area. It resembles the one in *A. rigidum*, but it is somewhat narrower than in this species (Fig. 9H, I). The structure is as in *S. scalpellum*, relatively oval and deep, and is located near the tip of the adductor muscle, but this receptacle normally houses more males than the latter species (Fig. 9G–I).

Finally, we found a narrow and small sleeping bag-like morphology in *Anguloscalpellum angulare* (Fig. 10A–C). We could not digest scuta of this species since it is rare museum material, but LM confirmed that only one male is present in the receptacle at a time (Fig. 10B), and that the shell-plate depression exhibits a morphology similar to other one-male, receptacle-type morphologies, being a narrow, deep depression in excess of 500 µm (Fig. 10B, C). The receptacle is preformed prior to the arrival of males, as adult, virgin specimens have a distinct depression in the receptacle area (Fig. 10C).

Weltnerium stroemii

As already found by Buhl-Mortensen & Høeg (2006), the receptacle area is located close to the transition to the tergum (Fig. 11D–F). In this area, the scutum has a depression that develops through three distinct stages (see discussion on ontogeny below) before achieving a final shape, which is exactly as found in *W. gibberum*. Again, this depression fits only the size of a single male (Fig. 9C).

THE ONTOGENY OF THE RECEPTACLE

We studied the ontogeny of receptacles in *W. gibberum*, *W. stroemii* and *S. scalpellum*, since for these we had sufficient numbers of both juvenile and adult specimens. Since there is never any clear specialization by external inspection, we focused on the structural development of the shell plate depression.

Scalpellum scalpellum

The minimum adult size is 7 mm capitulum length based on both our sample of many individuals and previous studies (Table 2; Buhl-Mortensen & Høeg, 2006; Spremberg *et al.*, 2012). To follow the ontogeny (see Fig. 5C, D), we examined specimens at 3 mm (juvenile) and 7 mm (adult) capitulum length. The 3 mm (juvenile) specimens have a shallow depression in the scutum shell plate in both SEM and µ-CT (Fig. 5C, D, F, I), whereas adult specimens have a deeper, more elongated depression, somewhat reminiscent of, but not identical to, the one found in *T. antillarum* (Figs 5C, D, G, H, 9I).

Weltnerium gibberum

The minimum adult size was found to be 5 mm capitulum length and we examined specimens of 3, 5 and 8 mm capitulum lengths. Within the last two size classes of adult specimens, we studied both specimens

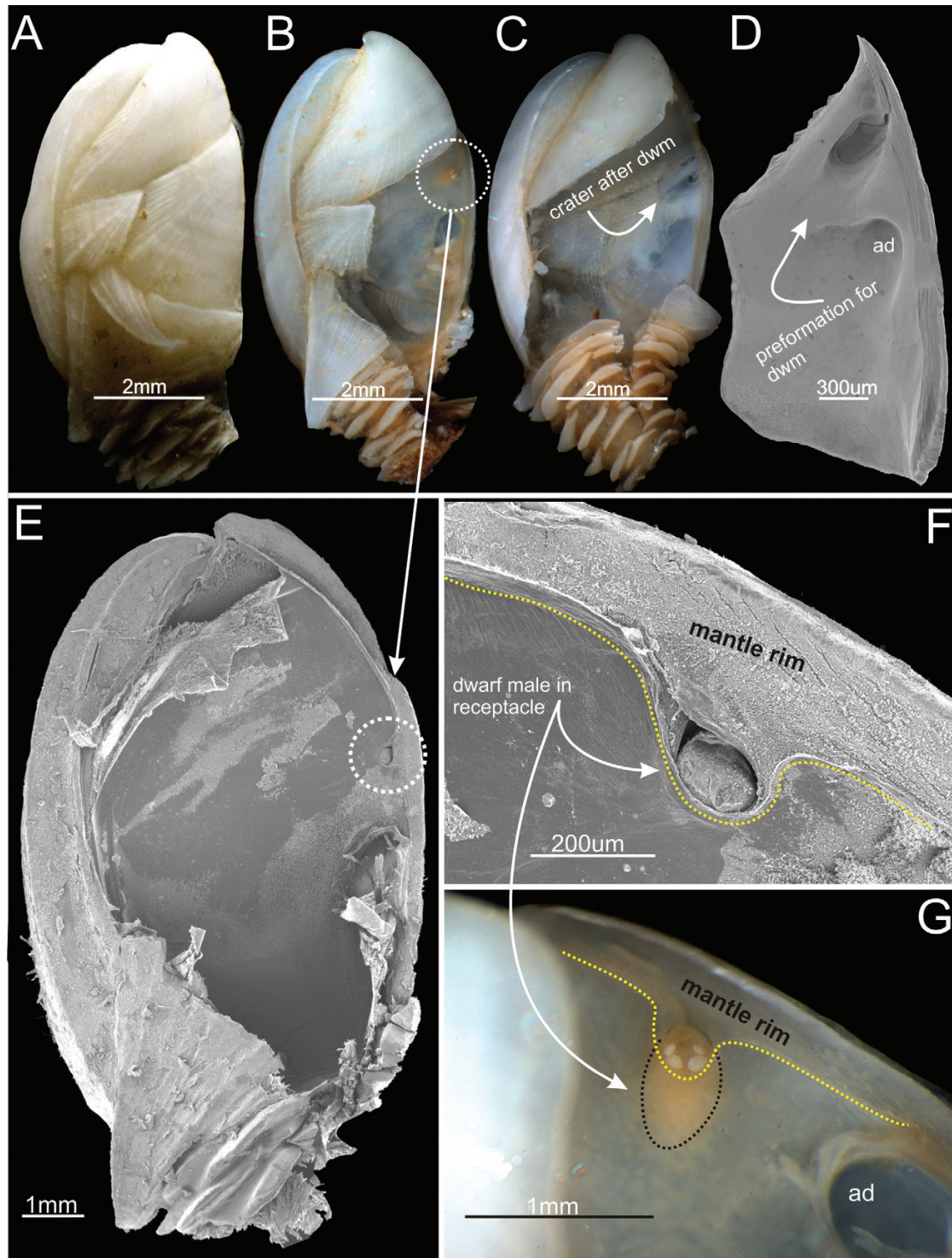


Figure 7. Receptacle morphology in *Verum brachiumcancr*. A, overview of adult specimen before dissection. B, removed left-side scuta exposing right-side scuta with a single dwarf male exposed in the receptacle. C, removed left-side scuta exposing right-side scuta with no dwarf male in the receptacle. D, SEM of a digested scutal shell plate revealing a very distinct preformation close to the transition to the tergum. E, SEM of specimen in (B), with the very small dwarf male exposed to the interior of the mantle cavity. F, close-up of (D), showing the single male attached in the receptacle. G, close-up LM of dwarf male in (B), showing the single dwarf male in the receptacle. Note how the ventral transparent lamella (vtl) bends medially where the dwarf male has settled. ad, adductor muscle.

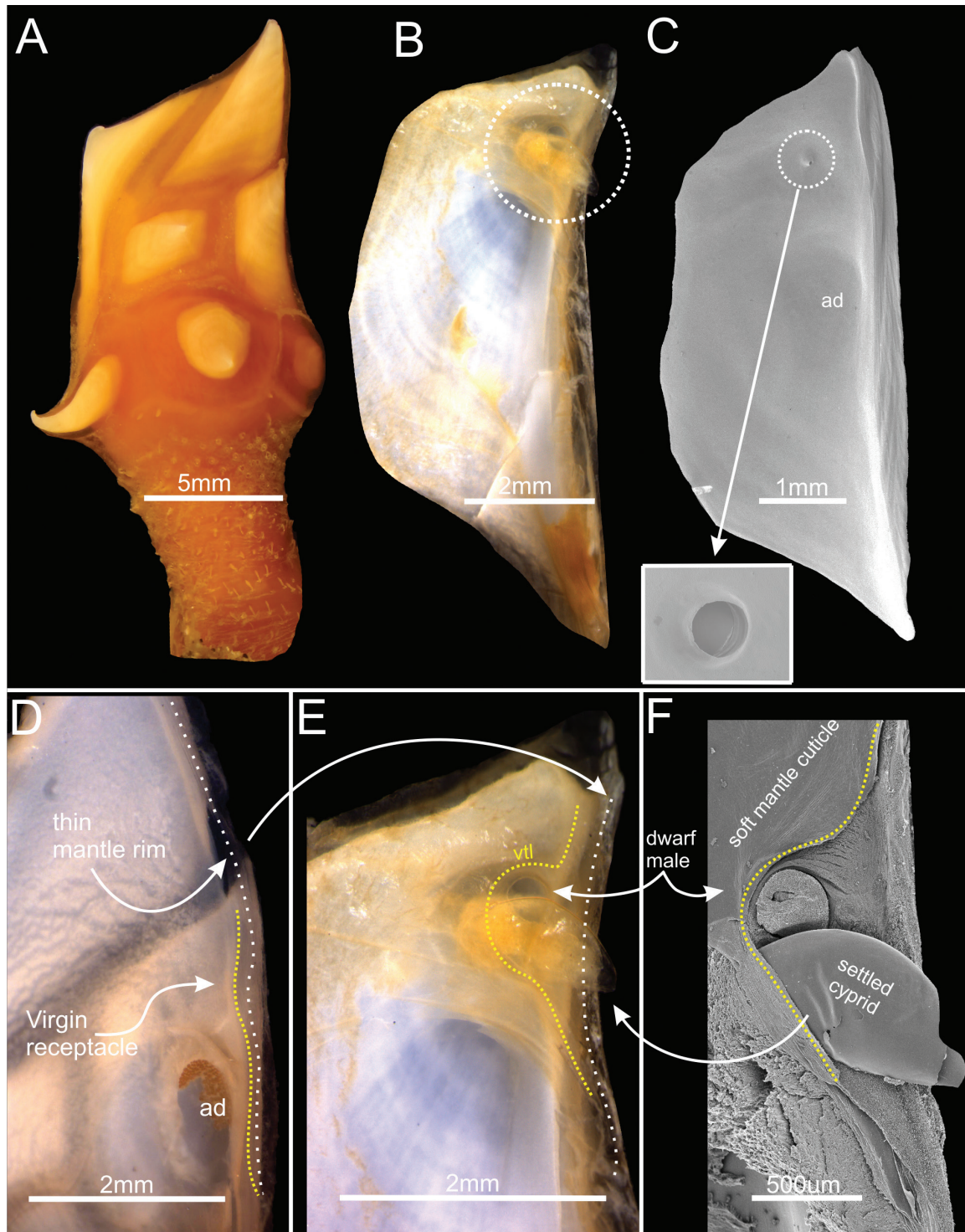


Figure 8. Receptacle morphology in *Weltnerium gibberum*. A, overview of adult specimen before dissection. B, right scutal shell plate with a cypris settled next to a dwarf male. C, SEM of a digested scutal shell plate. Note the deep and slender sleeping-bag-like preformation and the hole-like-structure apically in the receptacle preformation (insert). D, juvenile, virgin receptacle morphology with no external preformation in the shell plate or the mantle rim cuticle. E, close-up of (B), with a cypris and an already-settled dwarf male. F, SEM of (B), showing the dwarf male deeply buried, while the cypris is more exposed. Both are exposed to the interior of the mantle cavity. Abbreviations: ad, adductor muscle; vtl, ventral transparent lamella.

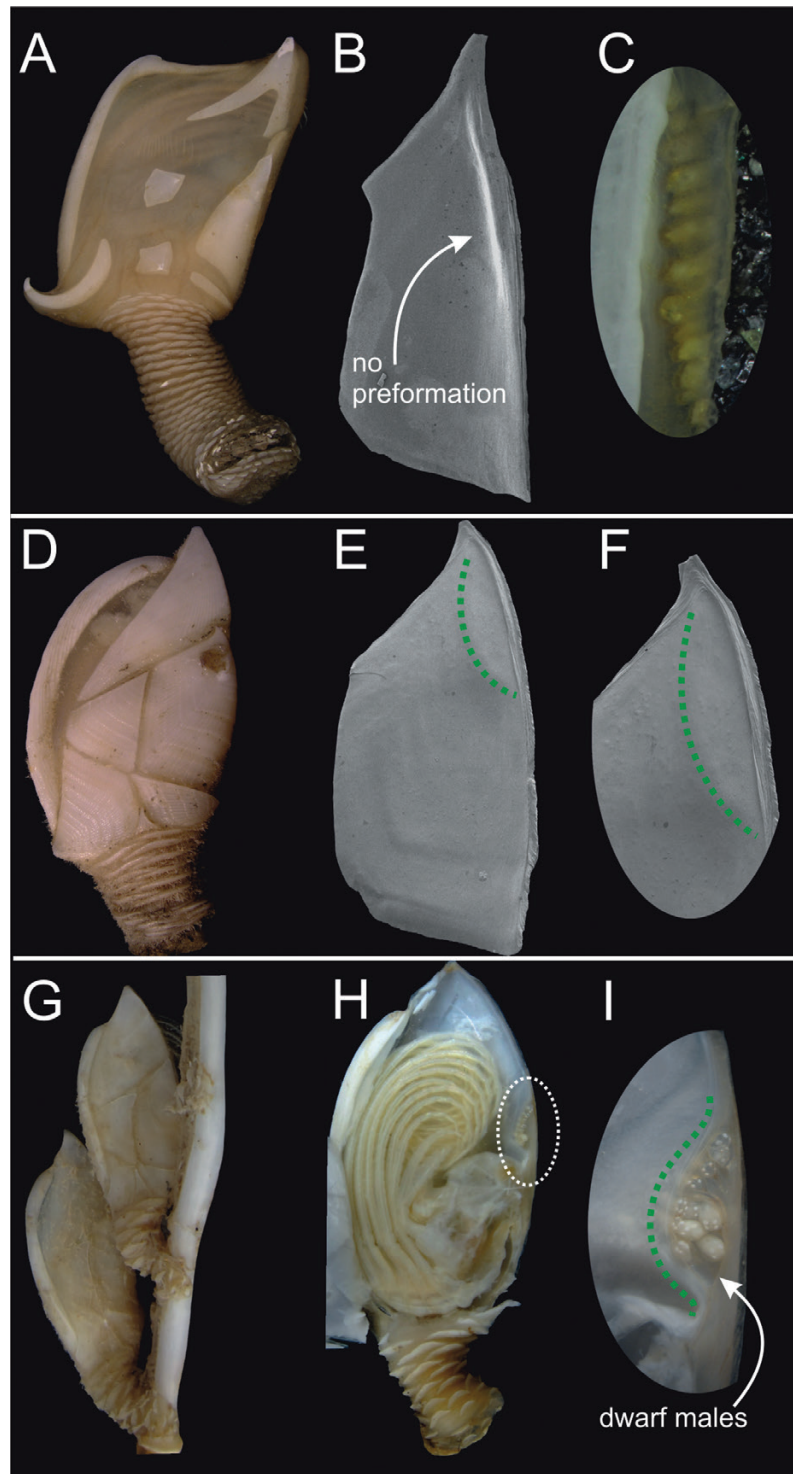


Figure 9. Receptacle morphology in *Scalpellum stearnsii*, *Amigdoscalpellum rigidum* and *Teloscalpellum antillarum*. A, adult *S. stearnsii* before dissection. B, SEM of digested scutal shell plate with no preformation. C, 1/3 of the total scutal shell plate exposing many males organized in single pits not creating a large, broad crevice as in other large scalpellids. D, adult *A. rigidum* before dissection. E, SEM of digested scutal shell plate with a narrow preformation close to the transition to the tergum (in green). F, close-up of (E). G, adult *T. antillarum* on unidentified spine. H, adult specimen with right-side opercular plates removed exposing right-side receptacle with many males. I, close-up of (H), exposing many males on top of shell plate depression (in green), which resembles, but is not identical to, the one seen in *S. scalpellum*.

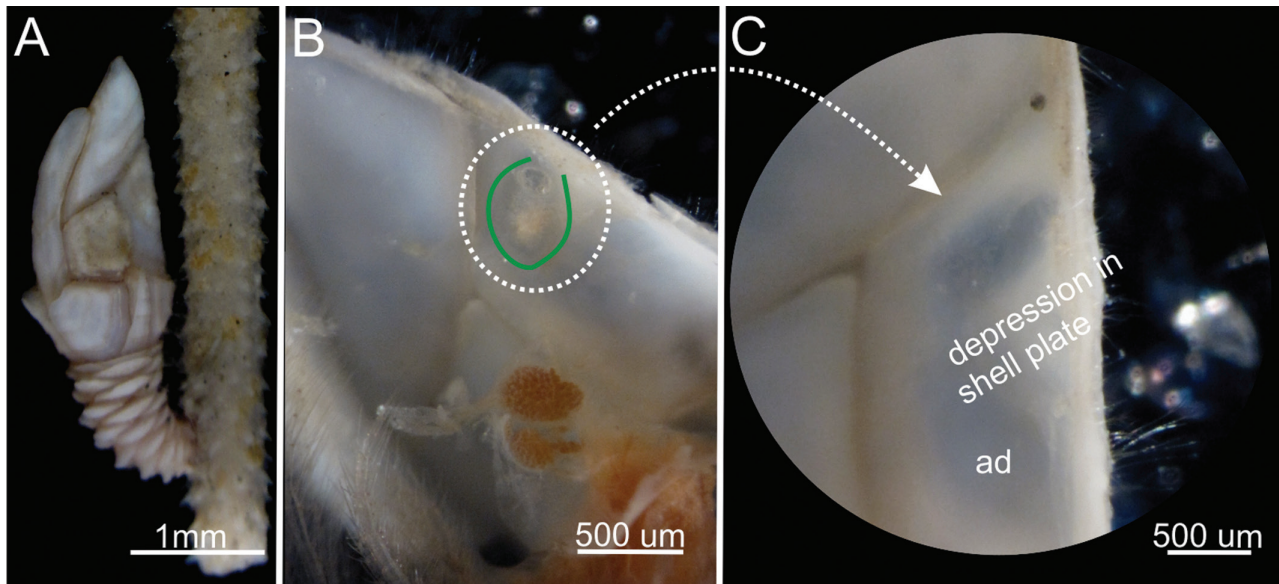


Figure 10. Receptacle morphology of *Anguloscapellum angulare*. A, overview of adult on unidentified spine. B, receptacle location and morphology with one dwarf male attached. C, zoom of receptacle in virgin adult with a deep preformation in the shell plate. ad, adductor muscle.

with males and virgin specimens (Fig. 11B, C). The virgin, adult females have the same depression in the scuta as the dwarf male carrying adults, indicating that this feature is preformed prior to the arrival of the cypris larva. Furthermore, there is no indication that the arrival of the male changes the shell plate depression in any way. During ontogeny, juveniles (3 mm) at first exhibit no structural preformation (Fig. 11A), but sub-adults (5 mm) have a broad and deep depression around the upper half of the scutum (Fig. 11B), which becomes narrower during subsequent development (Fig. 11C). In adult individuals at 8 mm, the depression has achieved its final sleeping bag-like morphology with narrow edges, as described above (Fig. 11C).

Weltnerium stroemii

Buhl-Mortensen & Høeg (2006) examined a large amount of material and found the minimum adult size to be 5 mm capitulum length. The receptacle area is located close to the transition to the tergum (Fig. 11D–F). In this area, the scutum has a depression that develops through three distinct stages before achieving its final shape at the adult stage (Fig. 11D–F). As in *W. gibberum*, there is no depression in small (3 mm), juvenile specimens (Fig. 11D). When reaching the minimum adult size of 5 mm capitular length, a depression has developed that is almost exactly as the one found in adults of *W. nymphocola* (Fig. 11E). In larger adults, the depression finally ends up having the shape seen in adults of *W. gibberum* (Fig. 11F).

REPRODUCTIVE DATA AND STATISTICAL ANALYSES

After scoring the reproductive data and dividing the receptacle morphologies of the species into three groups (see Table 2 and Discussion for details), we found that the sizes of females/hermaphrodites were largest in species with receptacle group 1, followed by group 2 and the smallest females/hermaphrodites in group 3 (Table 2). There was a significant relationship between the female/hermaphrodite size and receptacle type (multinomial logistic regression: $\chi^2 = 23.48$, d.f. = 2, $P < 0.001$). We also found that the number of males were largest in species with receptacle group 1, followed by group 2 and smallest in group 3, and this difference was again significant (GLM: $\chi^2 = 33.15$, d.f. = 2, $P < 0.001$). Although there was a tendency that dwarf male size differed between receptacle groups, this was not significant ($\chi^2 = 5.96$, d.f. = 2, $P = 0.051$). On the other hand, there was a positive relationship between the number of males and their size ($\chi^2 = 5.79$, d.f. = 1, $P = 0.016$).

DISCUSSION

RECEPTACLE MORPHOLOGY AND DIVISION OF GROUPS

We have given a comparative account of the morphology of the dwarf male attachment sites (receptacles) in 11 species of scalpellid barnacles and have compared this with data on the reproductive biology of the species (Table 2). Darwin (1851, 1854) was the

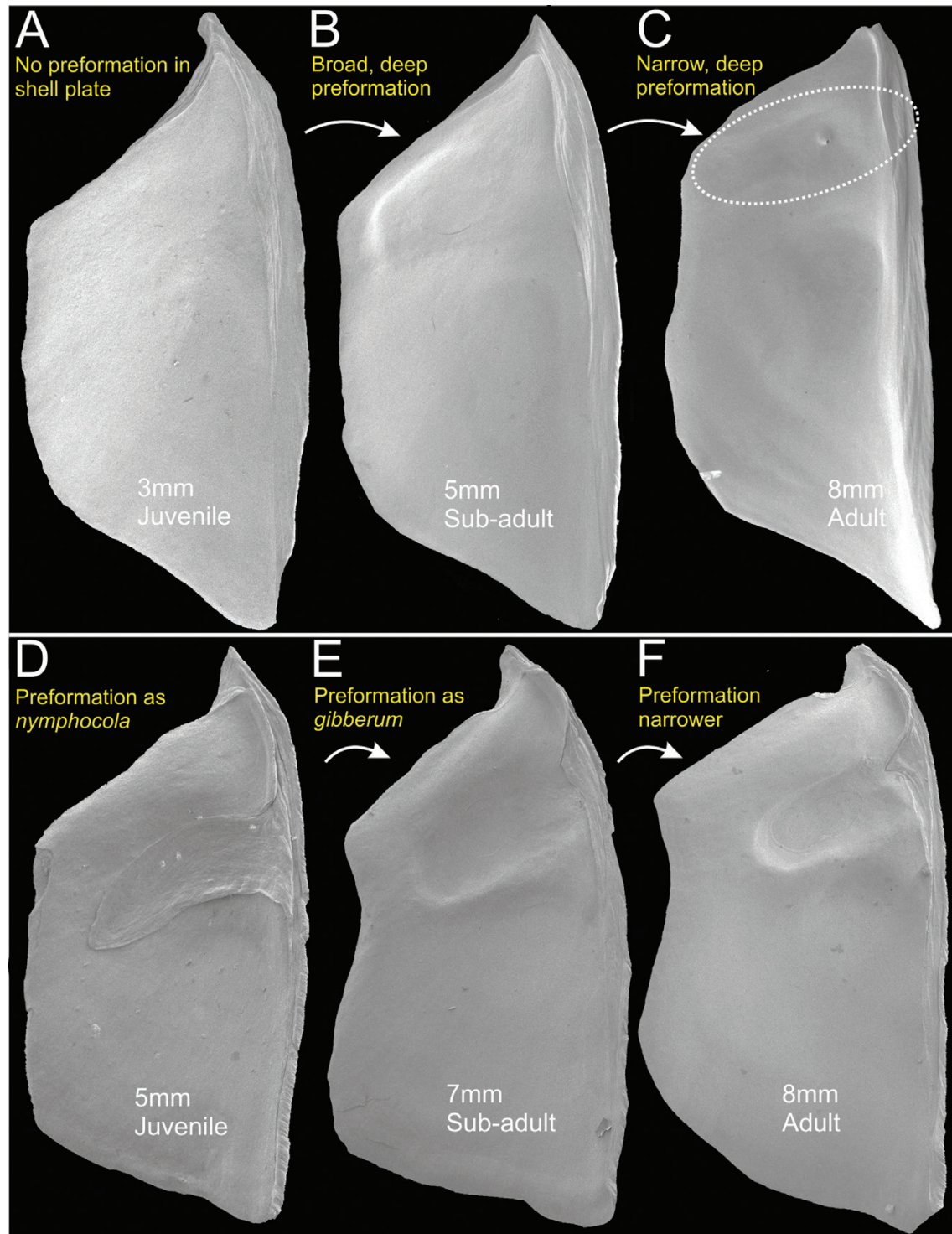


Figure 11. Ontogeny in *Weltnierium gibberum* and *Weltnierium stroemii*. A, B, C, ontogeny in *W. gibberum*, commencing with no preformation in juvenile specimens (3 mm) subsequently leading to a deep, broad preformation in the sub-adult stage (5 mm) terminating in the sleeping bag-like morphology in adult individuals (8 mm). D, E, F, ontogeny in *Weltnierium stroemii*, commencing with a stage like *W. gibberum* (not shown), subsequently leading through stages exhibited in phylogenetically less derived species: a *nymphocola*-like stage in juvenile individuals (5 mm) exhibiting a banana-shaped receptacle, a *gibberum*-like, broad, deep preformation in sub-adults (7 mm) and a smaller receptacle fitting but one dwarf male in adult individuals (8 mm).

first to study the receptacles of certain cirripede species and concluded that the morphology of this site was exclusively designed to receive males for better protection of the latter. Klepal (1987) compared the morphology of dwarf males across all cirripedes but did not discuss receptacle morphology in the females or hermaphrodites. While Lin *et al.* (2015) showed that dwarf males evolved by a single evolutionary event in the Scalpellidae, Gale (2016) constructed a phylogeny based on shell capitular morphology and included the first description of the depressions in the scutal shell plates of well-dated fossils. Buhl-Mortensen & Høeg (2006, 2013) extended these works in a handful of scalpellids, but our study is the first to use a standardized terminology and a more diverse methodical framework, together with an ecologically and morphologically wider range of species.

In all species examined in this study, the dwarf males were, irrespective of their number, located in two confined and symmetrically placed receptacles located on the rim of the mantle inside each of the paired scutal plates. Only *S. stearnsii* differed by having males situated all along the scutal plate (Fig. 9C). The receptacles vary considerably in size, numbers of males present and whether there is any structural preformation prior to arrival of the males. Investigation by LM and SEM revealed no externally preformed structures for the males, but some species have a distinct depression in the part of the scutal plate lying beneath the tissue of the receptacle area. The depression develops already in the juvenile stages and is present in virgin adults before arrival of the first males. We, therefore, conclude that such a shell plate depression is an adaptation serving to assist the settled cyprid to burrow deeper in the female/hermaphrodite tissue, thus gaining better protection than would be possible elsewhere on the mantle rim. Any dwarf male attaching outside the receptacle site will be more exposed and are, therefore, likely to experience a higher mortality due to, for example, mechanical damage. Thus, we interpret the receptacle area as a means by which the females or hermaphrodites can exert considerable control over the number of dwarf males they will eventually carry and the degree to which they are offered protection. This is supported by our observations on species such as *W. gibberum*, where the shell plate depression fitted only a single male, and there was no evidence that arrival of additional males led to a change in size of the receptacle structure.

Based on our examinations, we delineate three morphological groups of receptacles. These are outlined below.

Group 1 – no receptacle preformation

This group comprises *Arcoscalpellum michellotianum*, *Regioscalpellum regium* and *Scalpellum stearnsii*. These species probably exhibit a plesiomorphic

receptacle morphology without any preformation in the shell plates prior to the arrival of the males. This limits the extent to which the males can be protected by being deeply buried. Instead, they sit rather exposed to the exterior on the edge of the mantle rim, although usually within a large crevice. All evidence points to this being gradually formed by coalescence of individual holes formed by the males after they settle. In older females with large such crevices, the receptacle can hold numerous males, and newly arriving ones can profit from settling in empty spaces left by dead males without the need to burrow, and thus save time and energy.

All these species are among the largest scalpellids known and they presumably have a very long lifespan (years) in their cold and exclusively deep-sea habitats (Buhl-Mortensen & Høeg, 2006, 2013; Gale, 2016). The observation that newly settled cyprids can be found together with mature, old or even dead males shows that the females continuously acquire new mating partners to replace older ones that die after having exhausted their limited resources, and all along the receptacle depression continues to increase in size.

Group 2 – preformed medium-sized receptacle

This group comprises *Scalpellum scalpellum*, *Amigdoscalpellum rigidum* and *Teloscalpellum antillarum*. In these species, there is a shallow preformation in the scutal plates in the receptacle area, allowing a moderate number of males to burrow deep into the female or hermaphrodite tissue. Receptacles of this type will normally hold less than ten males, although larger numbers are occasionally encountered (Spremborg *et al.*, 2012). When many males are present, space limitations force some to the periphery in the receptacle area, where the plate depression is less deep. Accordingly, they cannot bury very deep into the tissue and end up having a larger part of their bodies exposed to the exterior. In *S. scalpellum*, we have even seen males attached slightly outside the receptacle area proper and, therefore, had their entire body completely exposed (Spremborg *et al.*, 2012; Høeg *et al.*, 2016).

Group 3 – preformed one-male receptacle

This group comprises *Verum brachiumcancrici*, *Weltnerium nymphocola*, *Weltnerium gibberum* and *Weltnerium stroemii*. The females of these species are generally very small and normally house one or two dwarf males per receptacle. As a direct result of a smaller body size, the cypris larvae settle and metamorphose on a much thinner sheet of tissue than in species of groups 1 and 2, but the highly specialized depression in the scutum enables a single one to bury deep down and thus gain maximum protection. The narrow but deep shape of the depression results in a very tight fit between male and female, and the location of the depression also entails that the dwarf males are functionally located inside the mantle cavity when

the valves are closed. This further protects the male and will facilitate retention of sperm during spawning. Our observations indicate that females can rely on the few first settled males for the duration of their lifetime and have no need to replace the first acquired ones. The absence of dead males in these species, and the scarcity of empty craters left by such ones, indicate that the females can rely on the first settled males for their entire lifetime. In agreement, newly settled cyprids adjacent to adult males were infrequent.

PHYLOGENY, ONTOGENY AND RECEPTACLE MORPHOLOGY

We did not incorporate a phylogenetic analysis in this paper as this has recently been put forward morphologically by Gale (2016) and molecularly by Lin *et al.* (2015). Using this phylogenetic information, our study indicates that phylogenetic constraints seem not to be the sole factor determining receptacle morphology among the analysed species. Our results clearly revealed that while females or hermaphrodites become smaller, their receptacle morphologies become more elaborate and advanced (Fig. 12). Further, we suggest that group 1 receptacles, with no shell plate preformation in large females, represent the original, plesiomorphic condition and that the narrow, elongate receptacles in females of group 3 are an advanced morphology (Fig. 12). Our group 3 species seem to be very closely related (Gale, 2016), so their narrow one-male receptacle could indeed represent an apomorphy that arose in the stem lineage to these scalpellids. It is also tempting to speculate that the group 2 receptacle is an intermediate evolutionary stage between groups 1 and 3. However, *S. scalpellum* (group 2) is phylogenetically more closely related to *S. stearnsii* (Group 1; Yusa *et al.*, 2012; Lin *et al.*, 2015; Gale, 2016). Nevertheless, *S. stearnsii* and *S. scalpellum* differ markedly in receptacle morphology, the sexual system, female/hermaphrodite size and the number of males carried by these (Fig. 12; Buhl-Mortensen & Høeg, 2006; Ozaki *et al.*, 2008; Spremberg *et al.*, 2012). Therefore, the group 2 receptacle, as exhibited in *S. scalpellum*, must be a condition that arose from within group 1 species and independently of any pathway leading to our group 3 species, thereby emphasizing that receptacle morphologies are not constrained by phylogenetic relationships (Fig. 12).

The morphological ontogenies of the receptacles reflected the evolution of the structural preformation and thus seem to indicate that phylogenetic character information can reside in the development of receptacle morphologies. In relation to questions posed on dwarf male evolution, it is of great interest to establish to which extent females or hermaphrodites are

structurally predestined to facilitate dwarf male settlement and, subsequently, their development. It further adds to the value of comparing sexual systems in cirripedes that extreme examples of such an adaptation is found in some parasitic cirripedes (Høeg & Lützen, 1995), where the dwarf males are nourished by, and fully integrated into, the female tissue. In this study, we have for the first time pursued this question in scalpellid cirripedes by analysing the ontogeny of three selected species, *Weltnerium gibberum*, *Weltnerium stroemii* and *Scalpellum scalpellum*, in all of which the ontogeny of the shell plate depression found in the receptacle proceeded rather differently. In *S. scalpellum* (group 2), a small, but distinct depression appears already in juvenile individuals (<7 mm), and it develops gradually into the deeper, oval shape found in adult hermaphrodites (Fig. 5C, D). In both *W. gibberum* and *W. stroemii*, the juvenile females first pass through a stage with no visible preformation, before developing the narrow sleeping bag-like depression in the scutum characteristic for adults of these species (Fig. 11A–C). It is furthermore interesting that the depression in *W. stroemii* consecutively passes through shapes resembling first *W. nymphocola* and then *W. gibberum* before assuming its final species-specific morphology (Fig. 11D–F). It is unknown how other scalpellids develop receptacle morphologies, but future studies should seek to conduct such analysis, as these could be valuable in understanding the evolution of the sexual relationship between the dwarf male and partner in the Cirripedia.

THE RELATIONSHIP BETWEEN RECEPTACLE MORPHOLOGY AND REPRODUCTIVE DATA

Based on our morphological analysis, we statistically analysed the relationships between receptacle group, female/hermaphrodite size, and the number and size of males (Table 2). In summary, the large species have no structural preformation in the shell plates, and these spacious receptacles tend to host many males. Intermediate-sized and small species have receptacles of groups 2 or 3, respectively, with a more or less profound preformation in the scutal plate. Probably due to limitations of space in the tissue on the mantle rim, the smaller group 2 receptacles host only a few males, while the narrow group 3 receptacles normally host but a single male (Table 2; Fig. 12). A relation between female/hermaphrodite size and male number was also found in other scalpellids (Buhl-Mortensen & Høeg, 2006, 2013; Ozaki *et al.*, 2008) and in the lepadomorph cirripede *Koleolepas avis* (Yusa *et al.*, 2001). The relation between male numbers and size similarly agrees with Buhl-Mortensen & Høeg (2006, 2013). We failed to find a significant relationship between receptacle morphology and the sizes of dwarf males (see Results; Table 2; Fig. 13), although it might have been

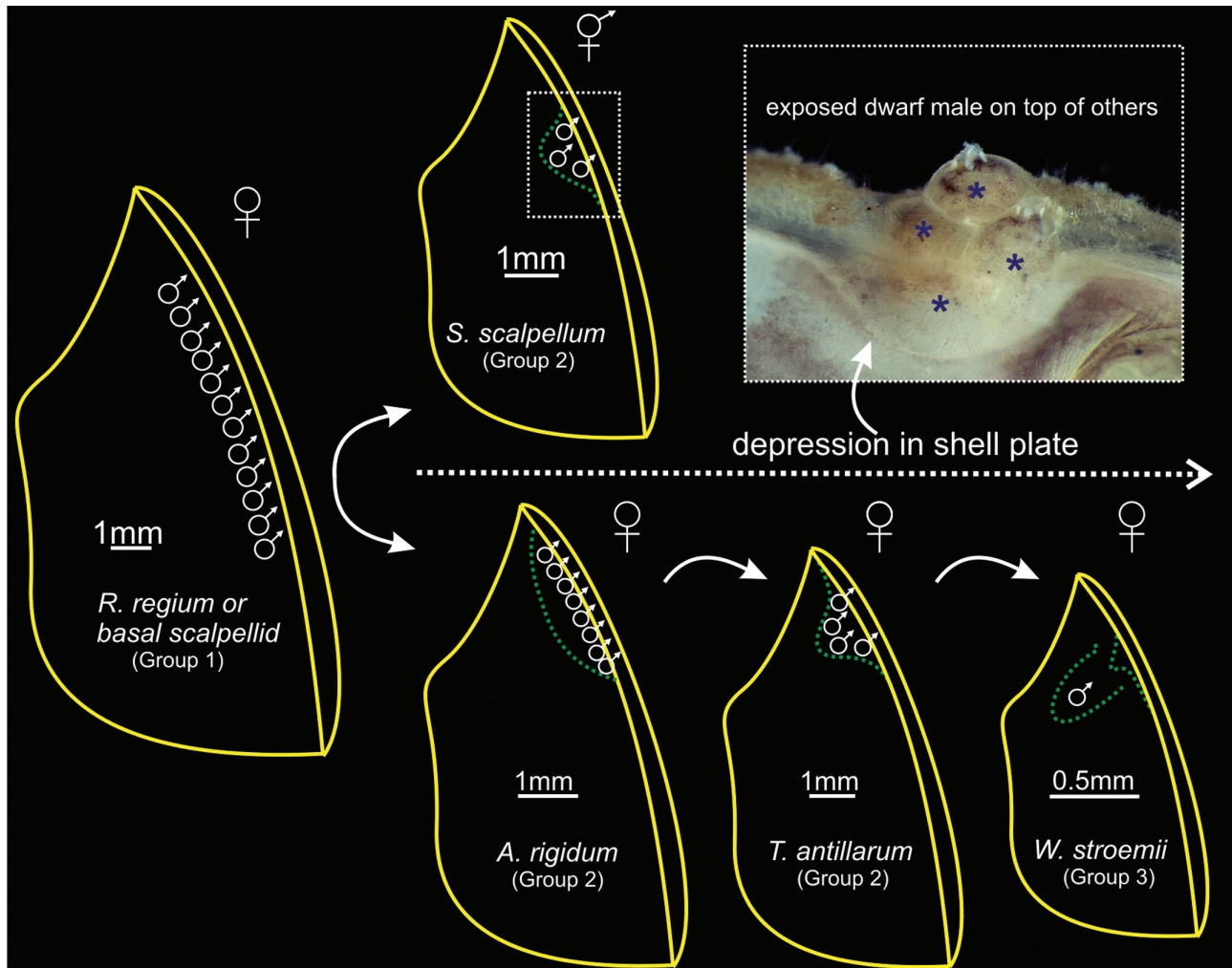


Figure 12. Overview of the receptacle evolutionary pathways in scalpellid barnacles. A large, basal scalpellid as *R. regium* (group 1) gives rise to two independently developed receptacle morphologies: the group 2 receptacles with a narrow, oval depression as seen in *T. antillarum* and *S. scalpellum* and the group 3 receptacles where females are gradually becoming smaller exhibiting more elaborate receptacle depressions, ultimately culminating in one-male systems as seen in *W. stroemii*. Because the receptacle in group 2 species is very narrow, later arriving males are forced to settle on top of each other (LM insert). Green and dotted lines indicate the scutal shell plate depression. Purple stars indicate dwarf males settled in the receptacle.

expected from the table given by Buhl-Mortensen & Høeg (2006). These data were taken from the literature and probably represent sizes of only a few or a single male for each species. Yamaguchi *et al.* (2007) analysed the growth of dwarf males in cirripedes theoretically and conjectured that dwarf males do not increase in size when the competition for sperm is low, such as occurs in our group 2 and 3 receptacles. In the latter, competition for sperm is relaxed, since each female normally hosts only a maximum of two rather small males. In contrast to the females of group 2, the large group 1 receptacles allow the males to be much more frequent, and the intensity in sperm competition among these males would favour them to be

larger in size in order to compete against sperm from other males. On the other hand, the often-numerous dwarf males in these receptacles may face space limitations due to crowding, which may again explain why we found no significant relation between receptacle type and male size. It would accordingly be worthwhile to analyse a larger sample of species in further pursuit of how male sizes depend on their number and the morphology of the receptacle.

We emphasize that the receptacle morphology is not obligatorily linked to number of males actually present. Females of our group 1 do not invest in the care of their males and their number on these females seems not to be limited by any other factor than the supply

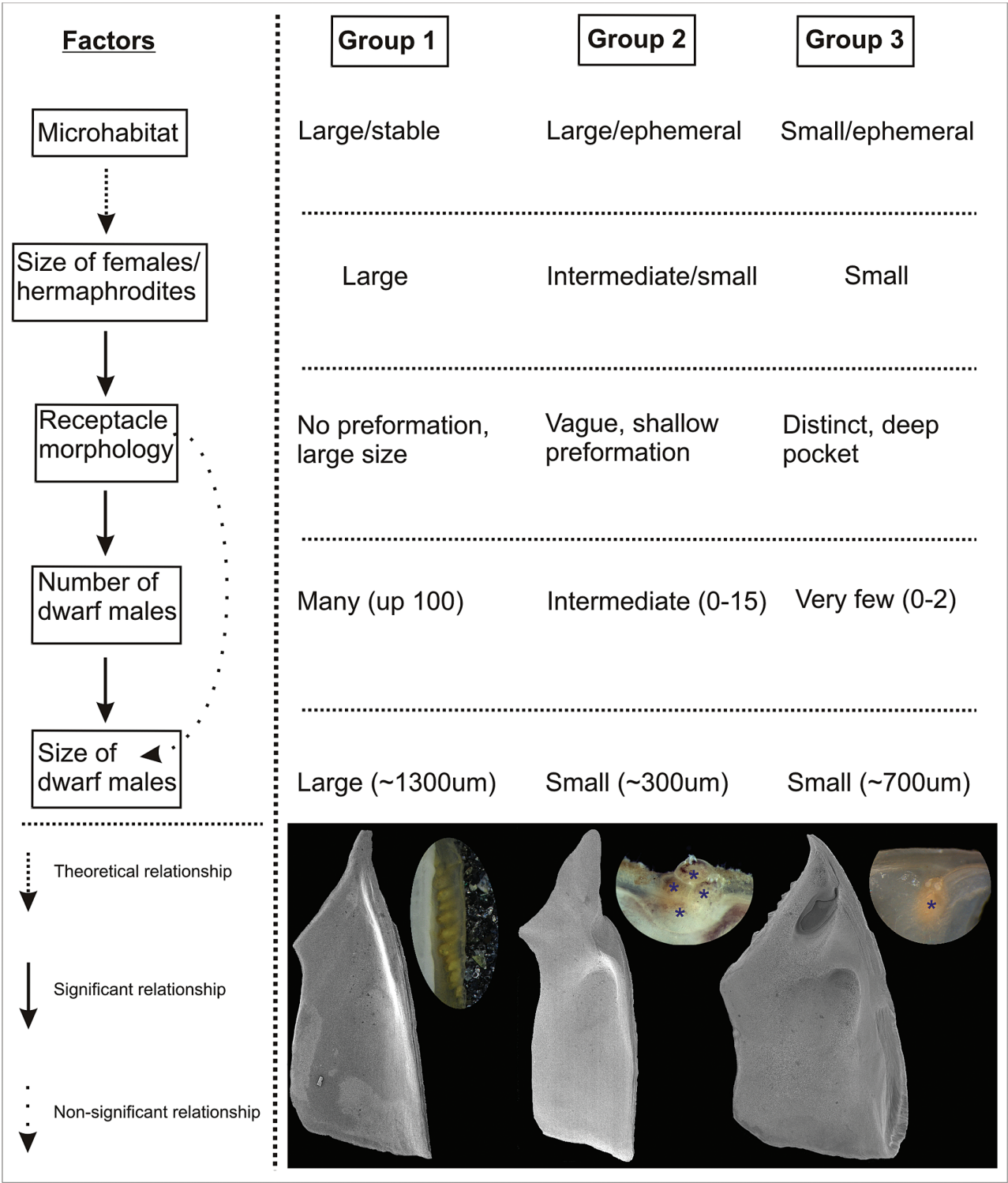


Figure 13. Summary of main findings on receptacle morphology and reproductive data. Above and below, left: flow diagram showing the (arrows) statistical relationships between the factors investigated in the study. Above, right: summary of findings on the factors in (above, left) based on groupings of receptacle types shown in the bottom (right) as *S. stearnsii* (many males, no preformation), *S. scalpellum* (fewer males, narrow, oval preformation) and *V. brachiumcancricri* (distinct preformation, one-male receptacle morphology).

of settling larvae and perhaps also the willingness of males to settle in an already competitive situation. In group 2 species, such as *S. scalpellum*, a larger number of males entail that some will be peripherally located in the receptacle area (due to the structural morphology of the receptacle) and thus unable to burrow very deep down in the tissue (Figs 12, 13; Spremberg *et al.*, 2012; Dreyer *et al.*, 2017). Yet, males may actually sit on top of an already established and deeply burrowed one, probably preventing the latter from functioning (Figs 12, 13). Even in group 3 species, cyprids can occasionally settle outside the actual shell plate depression (Fig. 8E, F) and eventually mature into adult males, but such males will necessarily be much less protected, and we surmise that they may, therefore, also sustain a higher mortality than the first arrived male inside the pocket. In all these cases, males beyond the structural capacity of the receptacle inflict no cost on the female, as she does not provide these with any resources.

With respect to the strategy of the dwarf males, settling in a very suboptimal position may simply reflect a balance between a secure substratum and a high mortality if the cypris larva attempts to find another partner animal. The scalpellid dwarf male system is, therefore, very different from that seen in the parasitic barnacles (Rhizocephala), in each of which a single male is provided with food resources. Here, the female has various mechanisms to restrict the number of invading males, while the first two arriving males block subsequent invasion into the receptacle of later arriving ones (Høeg & Lützen, 1995). The Rhizocephala, therefore, presents a rather unique example of lifetime partnership between male and female, and a most interesting platform for comparing reproductive strategies and the evolution of mating systems in the Cirripedia.

MICROHABITATS, LONGEVITY AND SETTLEMENT OF DWARF MALES

We argue that the different receptacle types, their morphologies and, ultimately, how many males these are capable of carrying may be a consequence of the species occupying different habitats inflicting the life-history traits (Table 2; Fig. 13). The microhabitats may restrict both the maximum size and the longevity of the females/hermaphrodites. In the deep sea, rocks, sea mounts or long-lived and hard-shelled invertebrates offer stable substrata on which the females can sustain a considerable longevity and, therefore, reach a large body size (group 1). In contrast, surfaces of many other organisms used by scalpellids (hydroids, arthropods and polychaete tubes) are rather ephemeral, whence individuals attached to these tend to have a short life-expectancy. These females or hermaphrodites are directly affected by, for example, the moulting cycles

of their 'hosts' and are, therefore, limited to a small body size, such as in our group 2 and 3 species (Table 2; Fig. 13; Buhl-Mortensen & Høeg, 2013; Ewers-Saucedo *et al.*, 2016). There is little information on growth and lifetime expectancy in scalpellids, but our observations on live *S. stearnsii* and *S. scalpellum* indicate a similar pattern. Where the former species lives for many years, the latter seems rarely to attain an age of more than 2–3 years (Ozaki *et al.*, 2008; Dreyer *et al.*, 2017).

Scalpellid dwarf males are always lecithotrophic and thus contain limited resources for sperm production over time. Large females, as those seen in group 1, with a considerable life-expectancy must, therefore, continuously acquire new dwarf males to assure continued fertilization of their large broods (~1600 eggs per brood; Buhl-Mortensen & Høeg, 2006). They, therefore, depend upon a specific receptacle size and morphology that allows the simultaneous presence of many males, such as actually seen in these females, where the number of males are up to 100 (Ozaki *et al.*, 2008; Fig. 13). On the other hand, they need not invest in any particular protection of their males (i.e. a structural preformation in the receptacle area), since they always carry enough to assure a reliable sperm source. A species with an intermediate life-expectancy, such as *S. scalpellum*, has a receptacle that allows some continued recruitment of dwarf males, although they rarely carry more than a few at any time (Table 2). These males are offered moderate protection by the relatively shallow receptacle that often leaves later arriving males rather exposed at the periphery of the area (Fig. 12; Spremberg *et al.*, 2012; Dreyer *et al.*, 2017). Finally, small and short-lived species on ephemeral substrata will need only a couple of dwarf males during the entire lifespan for fertilization of their few and small broods (~100–250 eggs per brood; Buhl-Mortensen & Høeg, 2006). But being wholly dependent on these few males, they have invested in a structural preformation of the receptacle area that offers maximum possible protection for one male at either side (Figs 12, 13). This relationship approaches the very advanced one found in the parasitic cirripedes of the Rhizocephala, although group 3 scalpellids never provide nutritional resources to their males. We argue that the general ecology and life history of scalpellid species have forged the adaptive evolution of diverse female/hermaphrodite strategies in terms of how much they invest in the caring for their dwarf males.

As a final comment, our structural study cannot directly explain why cyprids settle only in two restricted areas on their large partner, even in the absence of any structural preformations, such as is the case in our group 1 species, although this site might be more adjacent to the oviposition site in the partner. It would be interesting to study whether the settlement of dwarf males in two perfectly located areas is due to a localized chemical

stimulus, such as a SIPC compound (settlement inducing protein complex) that is now known to be responsible for the gregarious settlement behaviour found in many barnacles (Aldred & Clare, 2009). In lepadomorph species with dwarf males there is no structural settlement site prepared by the partner for the latter and the cyprids have no such preferred settlement site but attach on the general outer surface of their partner (Yusa *et al.*, 2010; Lin *et al.*, 2015; Wijayanti and Yusa, 2015; Dreyer *et al.*, 2018).

CONCLUSIONS

This paper has examined the morphology and evolution of the receptacles in which scalpellid dwarf males are almost invariably attached. We have demonstrated significant species-specific variations in the number of males and correlated this to the presence of preformed shell plate depressions in the female or hermaphrodite partner animal. When present, these depressions facilitate the development of the dwarf males and serve to provide the female or hermaphrodite with a more or less precise control over the number that will be successfully recruited. Species with few males show the largest investment towards their males in offering maximum morphological protection for normally only two males. We suggest that the variation in receptacle morphology and male size and numbers are ultimately correlated to the general ecology and habitat of the species. Additional support for our hypothesis will require much more data on the detailed life histories of a larger sample of species and including also embryo size and clutch size. Such an analysis should, as ours, also include a phylogenetic perspective.

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